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Sound speed and density characterization of milk adulterated with melamine

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Abstract: Milk contaminated with melamine resulted in an important health hazard that affected many babies in China recently. Ultrasonic characterization of adulterated milk may detect gross levels of melamine contamination. Sound speed and density measurements were made in skim milk as a function of melamine adulteration. An ultrasonic measurement technique to implement milk quality control is discussed.

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PACS numbers: 43.20.Hq, 43.35.Bf, 43.58.Dj [AN]
Date Received: December 17, 2008 **Date Accepted:** March 3, 2009

1. Introduction

Shortly after the conclusion of the 2008 Olympics, international health alerts were posted in response to milk adulteration in China. That adulteration resulted in kidney illness of varying degrees affecting, according to some reports, about 300 000 babies, 6 of whom died. In the global economy, food products often contain ingredients coming from different countries, which make quality control difficult. Although international regulations try to assure the safety of foods all over the world, this illegal, intentional contamination provides compelling evidence that better, reliable techniques are needed to guarantee the safety of foods we consume.

In the particular case of the tainted milk cited above, adulteration was not detected because the traditional milk quality sensors that were used were based on nitrogen quantification as an indirect method to determine protein content.[1] Although water was added to milk to fraudulently increase the productivity, melamine, which is an organic molecule rich in nitrogen, was added too, hiding the milk adulteration. Following Barbano and Lynch,[2] the development of multi-sensor quality sensing devices, based on the measurement and detection of different parameters, would improve the odds of detecting fraud. This is not so different in principle from an ordinary person checking a suspicious beverage by smelling, tasting one small sample, viewing the color, or jostling the beverage to intuitively judge viscosity or density or some other characteristic.

Ultrasonic non-destructive assays are commonly used in industry as reference techniques to assess the quality of many products and detect incipient or eventual failures in structural elements, materials, and processes. Ultrasound is widely used in industrial process control, e.g., in measuring the flow velocity of liquids, gases, and some mixtures, also liquid level and in other specialized analytical measurements such as composition of mixtures. Many industrial applications benefit from the transducers being external to the boundaries of the process. In NDT/NDE, e.g., thickness gauging or weld inspection, the interrogation is non-destructive even when the medium must be contacted by the transducers. Medical uses of ultrasound are familiar in clinics and hospitals around the world. Nevertheless there is limited application of ultrasound in the quality control of foods, drugs, and biological media, where chemical or biochemical techniques are commonly preferred. Recent developments are showing that ultrasonic-based sensing systems are well suited to achieve quality control in these

industrial sectors,[3] and research is being conducted for the control of fermentation and gelation processes, enzymatic reaction monitoring, or microbiological growth detection in milk.

Ultrasonic techniques have been implemented in non-invasive on-line measuring systems. They can avoid the important hazard of product contamination, with the advantage of making real-time corrections in the production chain and may permit easy automation of quality control. Another important feature of ultrasonic measuring techniques is that, in most cases, neither reactive nor replaceable elements need to be added to the medium under test. This implies that the ultrasonic inspection and quality control system may be implemented in an environmentally friendly and economical manner. On the other hand, ultrasonic inspection of milk based on sound speed c is subject to interfering variables (temperature, fat content, etc.) that can mask detection of an adulterant such as melamine.

The European Food Safety Agency[4] (EFSA) states a limit of 0.5 mg/kg body weight for a tolerable daily intake (TDI) of melamine. Nevertheless, melamine concentration in milk up to 2.5 g/kg was found in the cited adulteration, exceeding the TDI by a factor of 5000. In the present work, it is shown that acoustical characterization of tainted liquid milk, through density and/or sound speed measurements, can be used to detect gross melamine contamination, provided uncertainty in interfering variables such as milk fat or temperature does not mask m, the melamine concentration in adulterated milk. To the extent that an ultrasonic inspection can be implemented more easily and economically than alternatives, it might be a worthwhile first step in screening. Products passing the ultrasonic test might still contain dangerous levels of contaminant below ultrasonic detection limits. Therefore, products which get a passing grade from the initial screening ought to be subjected to other tests (e.g., mass spectrometer) that can reliably detect smaller but nevertheless potentially hazardous concentrations, even though such subsequent tests might be more complicated or more expensive to conduct on-line or in samples of batch-produced product, compared to a clamp-on or non-invasive ultrasonic test.

2. Experimental setup

2.1 Sample preparation

A liquid mixture was prepared containing distilled water, lactose, and melamine. The proportion used was 47 g of lactose and 8 g of melamine for each kilogram of water. This liquid has the same lactose concentration as milk. The melamine concentration was adjusted to reach the same nitrogen level as ordinary milk. Adding such a mixture to milk maintains constant sugar content and nitrogen levels of the resulting liquid. Skim milk was used in the experiment to diminish the influence of fat dilution when the melamine mixture was added.

A magnetic stirrer with a heating plate was used to dissolve melamine and lactose in water. This mixture was maintained at 40 °C for the experiments to avoid melamine precipitation, as the solubility of melamine in water at room temperature, 20 °C, is 3.1 g/l. Then it was added to skim milk up to 30% by weight for the analysis. Using Eq. (1) the melamine concentration in milk, m, expressed in g/kg can be obtained as a function of the mixture addition M in percent:

$$m = 0.075M. \tag{1}$$

An amount of 2.25 g of melamine per kilogram of milk corresponds to 30% mixture concentration (Fig. 1). Although a 40 °C temperature was not necessary to dissolve 2.25 g of melamine in milk, the experiment was made at this temperature, to avoid precipitating the mixture before it was added to milk.

2.2 Measurement techniques

Milk samples were prepared containing different concentrations of the melamine-lactose-water mixture. A DMA 4100 Anton Paar density analyzer determined the density of the adulterated milk at 40 °C.

As the melamine mixture was continuously added to the milk, time of flight variations were measured using the ultrasonic device developed by Elvira *et al.*[5] The liquid sample was put

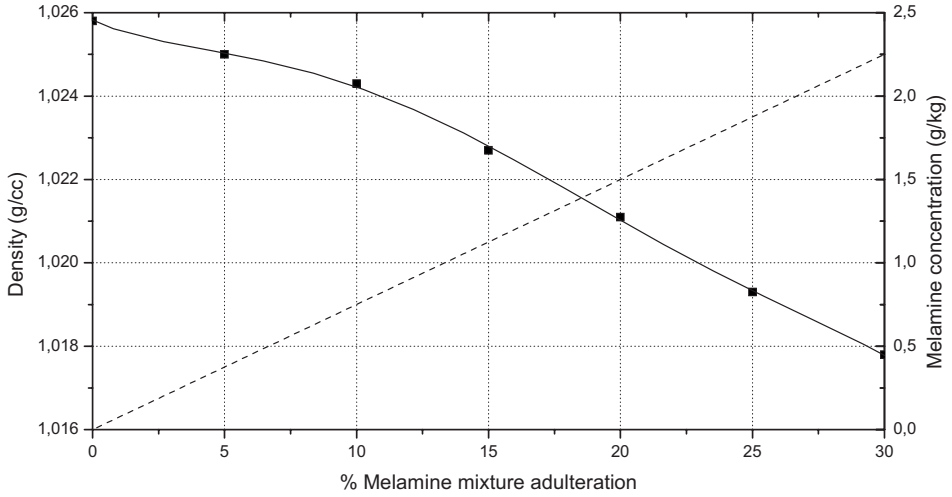

Fig. 1. Density and melamine concentration of the adulterated milk as a function of the melamine mixture concentration. Squares are density measurements and the solid line is a fifth order polynomial fit. Melamine concentration (dashed line) is obtained from Eq. (1).

in a 125 ml commercial glass bottle which was placed inside one of the ultrasonic measurement cells. The liquid path was 36 mm, traversed once. These cells are inside an environmental chamber. Each measurement cell consists of a thermostated housing with two ultrasonic transducers. Each consists of three PZT piezoceramic plates (PZ27 from Ferroperm). The transducers are placed on the opposite sides of the bottle and pressure-coupled through silicone layers when the housing door is closed. Temperature sensors, temperature actuators (Peltier cells and resistances), and control electronics based in PID (proportional-integral-derivative) controller devices are used to avoid specimen temperature variations greater than 0.02 °C. An efficient temperature control is necessary to obtain accurate measurements because sound speed is sensitive to temperature. For skim milk, near 40 °C, $dc/dT \sim 1.5$ m/s °C, close to the value for water.

The electronics to excite and receive signals is implemented in a PXI chassis (National Instruments). It is comprised of a ZTEC 530 Function Generator, a ZTEC 410 digitizer, a NI-2503 24 channel multiplexer, and a NI PXI-PCI-8336 MXI-4 module to communicate between the chassis, NI-1036, and a personal computer. The 4 MHz ultrasonic tone burst is captured after propagating through the liquid. From this through-transmitted pulse, variations in time of flight, resolved to ±1 ns (approximately 1 part in 23 000) were determined using fast Fourier transform signal processing. Sound speed c in skim milk at 40 °C was measured to be 1548 m/s. Therefore with this ultrasonic device, c changes as small as ±7 cm/s may be detected as a function of the melamine mixture added. In pure water at 40 °C, $c = 1528.863$ m/s.[6]

A peristaltic pump, Instech-720, was used to add the mixture to milk. Two tubes pass through the cap of the bottle for this purpose, one for mixture pumping and the other for pressure stabilization. The mixture was continuously added at a slow flow rate of 0.45±0.01 ml/min to avoid temperature changes in the sample. The mixture, kept inside the climatic chamber, was maintained at the 40 °C working temperature.

3. Results and discussion

Density measurements were obtained at 5% mixture intervals. As can be seen in Fig. 1, a clear decrease in density was obtained. It is mainly due to the dilution of milk. True milk has a 32 g/kg concentration of proteins which is higher than its substitute (melamine) which was adjusted to 8 g/kg in the adulterating mixture. The density variation as a function of melamine is even more significant when the melamine concentration is more than 10%.

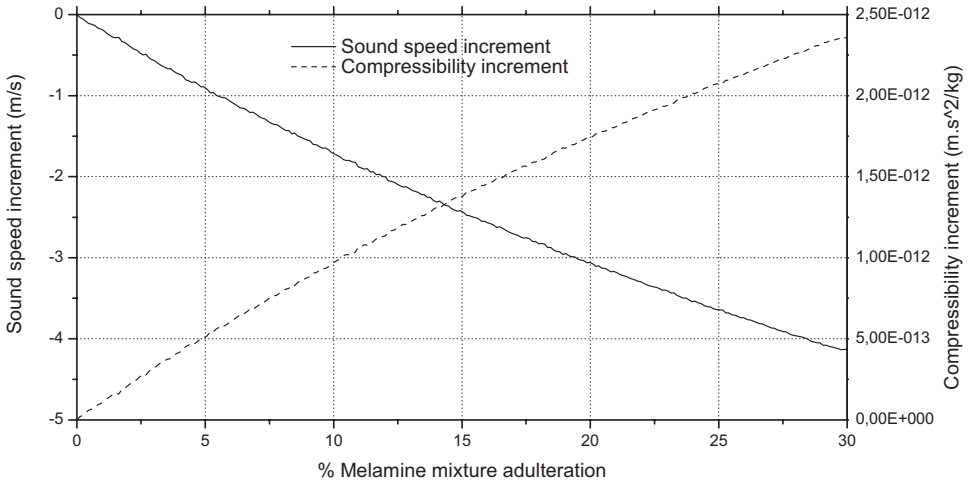

Fig. 2. The solid line shows the sound speed variations as a function of the melamine mixture concentration. Dashed line is the compressibility calculated from sound speed and density measurements.

When the milk was analyzed in the ultrasonic characterization device, an important *decreasing* of sound speed up to 4 m/s was registered (Fig. 2). The measured density decrease, at constant compressibility, would have produced a velocity increase, so the measured sound speed diminution means that the isentropic compressibility of the adulterated milk increases. This increase is shown in the dashed line which was computed from the measured density and the sound speed data (Fig. 2). As stated above, the liquid obtained when the mixture was added has a lower solute concentration. This lower concentration is responsible for both the compressibility increasing and the density decreasing. The c data for melamine in milk are believed to be new. It is reasonable to think that an adulteration made to obtain an economic profit by a fraudulent increasing of milk volume must incorporate a significant amount of melamine mixture, as occurred in this recent case. Ultrasonic screening, to the extent it is inexpensive, robust, and easy to implement, might prove to be a worthwhile quality control method for milk.

From data in Fig. 2 and the literature,[5,6] an equation relating c in melamine-adulterated skim milk to melamine mixture concentration M in percent and temperature T near 40 °C may be derived, for M up to 30% by weight:

$$\Delta c = AM + BM^2 + C\Delta T, \tag{2}$$

where $A=-0.1873$ m/s, $B=0.001\,65$ m/s, and $C=1.5$ m/s °C. Δc is in m/s and ΔT is in °C.

According to EFSA,[4] a harmful concentration for babies will be near 0.003 g of melamine/kg milk, which means $M=0.04\%$ from Eq. (1). This will increase transit time across a 36 mm liquid path by 0.1 ns and reduce c by 7 mm/s. Following Eq. (2), the same c reduction would occur for a T reduction of 0.005 °C. A small uncertainty in fat content or other composition factor is also likely to mask 3 mg of melamine/kg milk. However, if c uncertainties due to T, fat, or other variables amount to <1 m/s, and if c can be measured online to 0.1% accuracy (±1.5 m/s), then total uncertainty in sound speed will be <2.5 m/s. From Fig. 2, this speed detection limit corresponds to a minimum detectable value for M of 15%. It appears reasonable that 15% melamine adulteration can be detected on-line. Even though this level, $M=15\%$, is substantially above the stated TDI minimum harmful concentration ($M=0.04\%$), detecting at the $M=15\%$ level would have been sensitive enough to detect 30% gross adulteration as reached in the recent milk contamination.

Relationships among sound speed, density, and protein concentration have been noted previously in a liquid which is substantially different from milk, namely, blood at 37 °C.[7] Ul-

trasonic thickness gauges and ultrasonic transit time (contrapropagation) flowmeters operating at or above 1 MHz typically resolve transit times or transit time differences to subnanosecond precision. In some transit time *ultrasonic flowmeters*, the sound speed has been used to determine concentration of a binary mixture.[8] Sometimes *ultrasonic thickness gauges* are used to test specimens of known thickness, yielding sound speed in the specimen, which in turn may be related to elastic moduli, alloy type, or other compositional aspect. This industrial experience suggests that commercially-available NDT or flowmeter process control instrumentation might be adaptable to achieving reliable on-line resolution of transit time comparable to that obtained in the laboratory with the present equipment. However, limits on using c to determine m or M probably come from interfering variables, rather than from the precision of a c determination.

In the laboratory there is ample opportunity to determine the wall thickness and wall transit time of the vessel and eliminate its uncertainty from the measurement of sound speed. On-line, uncertainty in wall thickness might limit accuracy of clamp-on sound speed measurements. Differential path solutions[9] may obviate such on-line clamp-on potential problems. While not investigated in the present study, one might utilize scattering, attenuation, or received amplitude[5] as the basis of further ultrasonic methods to detect undissolved adulterants such as settled melamine powder in closed vessels. The practicality of an on-line ultrasonic detection system for screening or other purposes is limited in part by contributions to c by interfering variables. Quantifying the influence of fat content and other contributions to c as would be encountered during normal milk production appears to be the logical next step.

4. Conclusions

Melamine powder, dissolved in water and added to skim milk at 40 °C, to create an adulteration concentration of 30%, decreases the density of the mixture by about 0.78% and decreases the mixture's sound speed by about 0.25%. Sound speed c was determined in the present experiments with resolution of about 1 part in 23 000 using a through-transmission liquid path of 36 mm, traversed once, and timed to ±1 ns. Absent interfering uncertainties due to temperature T, fat content, or other factors, resolution of c to ±1 m/s corresponds to 6% melamine mixture at minimum concentration. However, even with c measured on-line to 0.1% accuracy, if uncertainties in T or other factors lead to ±1 m/s contributions to c, then melamine concentrations below 15% are likely to be masked. While the practicality of an on-line ultrasonic detection system for screening or other purposes is limited in part by contributions to c by interfering variables, an ultrasonic technique capable of detecting 15% melamine concentration would have been sufficiently sensitive to detect gross adulteration at the 30% level reached in the recent milk contamination (2.5 g of melamine per kilogram of milk). It is reasonable to think that an adulteration made to obtain an economic profit by a fraudulent increasing of milk volume must incorporate a significant amount of melamine mixture, as occurred in this recent case. Ultrasonic screening, if inexpensive, robust, and easy to implement, might prove to be a worthwhile quality control method for milk.

Acknowledgment

The authors acknowledge Dr. David M. Barbano's comments about practical limitations.

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